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(54) Title: **USE OF ISOVALERAMIDE AS A MILD ANXIOLYTIC AND MILD SEDATIVE AGENT**

(57) Abstract

Isovaleramide has been found to exhibit mild anxiolytic (anxiety-reducing) activity at low to moderate dosage levels and mild sedative activity at somewhat higher dosage levels. In contrast to certain other materials thought to be anxiolytic or mildly sedative, isovaleramide is non-cytotoxic and does not paradoxically stimulate the central nervous system. Isovaleramide is therefore useful as a mild anxiolytic and mild sedative agent which can be made available to the general public.

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USE OF ISOVALERAMIDE AS A MILD ANXIOLYTIC AND MILD SEDATIVE AGENT

Background of the Invention

The present invention relates to an approach to reducing anxiety without producing undesirable excessive sedation in animal subjects, including humans. More particularly, the invention concerns the use of isovaleramide as a mild anxiolytic and a mild sedative agent.

The complex interrelationships of the receptors, neurotransmitters, and electrical impulses which affect the central nervous system (CNS) are far from being fully understood. However, it has been possible to distinguish the effects of various pharmaceuticals and other drugs on aspects of the central nervous system that are exhibited as behaviors.

In particular, it is possible to distinguish an anxiolytic (anxiety-reducing) effect from a sedative one, i.e., classical CNS depression. Standard behavioral tests, such as the exploratory behavior test and the Vogel Conflict Paradigm described below can be used to detect, demonstrate, and quantify the anxiolytic effects of various pharmaceutical agents. Other tests, including the prolongation of barbiturate-induced sleep time and the induction of sleep, show sedative and hypnotic effects, respectively.

While some agents are effective in inducing both anxiolysis and sedation, other agents, often structurally closely related, can be shown to exhibit primarily only one or the other activity. Moreover, the ability of useful antianxiety agents to exhibit primarily either anxiolytic or sedative activities is often related to dose. See Wolff, M.E. (Ed.) BURGER'S MEDICINAL CHEMISTRY, Part III, 4th ed. (1981) Wiley-Interscience, pp. 981-996; Foye, W.O. (Ed.) PRINCIPLES OF MEDICINAL

CHEMISTRY, 3rd ed. (1989) Lea and Febiger, pp. 143-237. Furthermore, there are additional demonstrable possible effects on the central nervous system such as the ability to induce or repress convulsions. Again, various
5 structurally related compounds may or may not exhibit these abilities. What is clear from the art available at present is that there seem to be no clear structure-function correlations that can be made with respect to predicting the ability of a particular substance to
10 affect or not affect the central nervous system in a prescribed manner.

The extracts of certain medicinal plants have been used for the reduction of stress and for the treatment of anxiety in many different cultures throughout the world
15 since time immemorial, and a number of anxiolytic and sedative principles have been isolated from higher plants and characterized in modern times. Indeed, benzodiazepine tranquilizer compounds such as diazepam ("VALIUM"), oxazepam ("SERAX"), and lorazepam ("ATIVAN"),
20 which have been considered to represent the quintessential synthetic anxiolytic agents par excellence, have now been shown unequivocally to be naturally occurring plant-derivable compounds, having been found in potatoes, soybeans, lentils, corn, wheat,
25 buckwheat, rice, oats, barley, and millet (Wildmann et al., *J. Neural Transm.* (1987) 70:383-398; Wildmann, J. *Biochem. Biophys. Res. Commun.* (1988) 157:1436-1443; Wildmann et al., *Biochem. Pharmacol.* (1988) 37:3549-3559; Unseld et al., *Biochem. Pharmacol.* (1989) 38:2473-2478;
30 Klotz, U., *Life Sci.* (1991) 48:209-215; Bringmann, G., J. *Neural Transm.* (1992) 88:77-82).

The use of valerian root extracts for medicinal purposes has centuries of history behind it, but the active sedative components have not been clearly or
35 positively identified (Krieglstein et al., *Deut. Apoth. Ztg.* (1988) 128:2041-2046), nor has the nature of the effect of the extracts been clearly characterized and segregated into behaviorally distinguishable effects.

The roots of valerian contain three principal classes of compounds: (a) the volatile oil(s), which are composed primarily of isovaleric acid and volatile monoterpene and sesquiterpene derivatives, (b) non-volatile
5 monoterpene iridoids or valepotriates, and (c) monoterpene alkaloids. The monoterpene alkaloids are only minor components and are not considered to contribute significantly to the effects of valerian; similarly, the volatile oil fraction seems only weakly
10 active. Attention has therefore focused on the valepotriate fraction, which is generally comprised of monoterpene (iridoid) esters. These esters are water insoluble, and cannot contribute entirely to the plant's sedative effect since aqueous extracts of valerian root
15 (presumably devoid of valepotriates) continue to exhibit sedative activity. The valepotriates may also be toxic (see below).

Recent studies on the valepotriates have shown that these compounds can irreversibly alkylate DNA and
20 proteins and that small quantities of orally administered valepotriates actually reach the mouse brain and other organs intact (Wagner, H. and K. Jurcic, *Planta Med.* (1980) 38:366-376). However, in spite of this potentially hazardous toxicity, the valepotriate
25 fractions have been marketed extensively as sedatives in Europe.

Aqueous and hydroalcoholic extracts of valerian root have traditionally been used as sedative preparations in Europe and the United States. However, ammoniated
30 tinctures of valerian root have also been used in the United States and Great Britain as sedatives, but only as crude mixtures. It is only from the ammoniated tinctures of valerian root that the inventors were able to identify and isolate isovaleramide, presumably a product of the
35 reaction of valepotriates with ammonia.

Isovaleric acid, isovaleramide and related substances are known to elicit narcotic and hypnotic effects in experimental animals when administered in very high

doses. See Eeckhout, A.v.d., *Arch. exptl. Pathol. Pharmacol.* (1907) 57: 338-57; Impens, E., *Deut. med. Wochschr.* (1912) 38: 945-47; May, P., *THE CHEMISTRY OF SYNTHETIC DRUGS*, 3rd ed. (1921) Longmans, Green and Co., p. 24; Meyer, K.H. and H. Hemmi, *Biochem. Z.* (1935) 277: 39-71; Junkmann, K., *Naunyn-Schmiedeberg's Arch. exptl. Pathol. Pharmacol.* (1937) 186: 552-64; Samson et al., *J. Clin. Invest.* (1956) 35: 1291-98; Teychenne et al., *Clin. Sci. Molec. Med.* (1976) 50(6): 463-72. Furthermore, salts of isovaleric acid were used as sedatives in the early twentieth century, but it was considered that their effect was psychological and due to the stench of the highly volatile free acid (Hare et al., *THE NATIONAL STANDARD DISPENSATORY* (1905) Lea Brothers and Co., pp. 94, 159-160, 1619-1620; Grier, J., *Chem. Drug.* (1929) 110:420-422; Allport, N.L., *THE CHEMISTRY AND PHARMACY OF VEGETABLE DRUGS* (1944) Chemical Publishing Co., pp. 159-161; *YEAR BOOK OF THE AMERICAN PHARMACEUTICAL ASSOCIATION*, 1912 (1914) 1:178-179; *DE RE MEDICINA* (1938) Eli Lilly and Co., p. 159). Isovaleramide itself was shown to exhibit hypnotic activity only when administered to experimental animals in very high doses and was considered to be clinically useless as a hypnotic. Impens, E., *Deut. med. Wochschr.* (1912) 38: 945-47; Junkmann, K., *Naunyn-Schmiedeberg's Arch. exptl. Pathol. Pharmacol.* (1937) 186: 552-64. A series of α -brominated and/or α -alkylated isovaleramide derivatives, however, were considered successful in this regard. See Volwiler, E.H. and D.L. Tabern, *J. Am. Chem. Soc.* (1936) 58: 1352-54; Burger, A., *MEDICINAL CHEMISTRY*, Vol. 1, (1951) Interscience, pp. 131-132; Burger, A. (Ed.) *MEDICINAL CHEMISTRY*, 2nd ed. (1960) Interscience, pp. 357-375; Burger, A. (Ed.), *MEDICINAL CHEMISTRY*, Part II, 3rd ed. (1970) Wiley-Interscience, pp. 1365-1385; Wolff, M.E. (Ed.) *BURGER'S MEDICINAL CHEMISTRY*, Part III, 4th ed. (1981) Wiley-Interscience, pp. 787-828.

Valnoctamide, a higher homolog of isovaleramide, is one such compound (see Figure 1). Valnoctamide is a

synthetic, barbiturate-derived, α -branched, water-insoluble compound which is purported to exhibit tranquilizing and anxiety-reducing properties which can quiet aggressive experimental animals and anxious humans (Stepansky, W., *Curr. Therap. Res.* (1960) 2: 144-47; Goldberg, M., *Dis. Nerv. Syst.* (1961) 22: 346-48; Roszkowski, A.P. and W.M. Govier, *Int. J. Neuropharmacol.* (1962) 1: 423-30), and which produces hypnosis in rats at doses of 250 mg/kg intraperitoneally (IP). Although valnoctamide may appear superficially to be structurally similar to isovaleramide, there are considerable physicochemical and pharmacological differences between the two compounds. For example, valnoctamide is very water insoluble, whereas isovaleramide is essentially water soluble. Furthermore, valnoctamide exhibits pronounced hypnotic properties in rats at relatively low doses (e.g., 250 mg/kg [1.75 mM/kg] IP), whereas isovaleramide is not hypnotic in mice even in doses as high as 1000 mg/kg (9.90 mM/kg) IP, and exhibits hypnotic activity only at near-toxic dose levels (2000 mg/kg IP and higher). Thus, the pharmacological profile of valnoctamide appears to more closely resemble that of its synthetic barbiturate precursor than that of isovaleramide.

Valnoctamide also differs from isovaleramide in that it contains two (chiral) stereocenters (the branched α and β carbons); isovaleramide possesses no (chiral) stereocenters. Thus, "valnoctamide" is actually a mixed racemic preparation consisting of four stereoisomers in two diastereoisomeric sets of enantiomers. It is not known whether all four of these stereoisomeric forms are pharmacologically equivalent. The present inventors have demonstrated, by means of carbon-13 nuclear magnetic resonance [^{13}C -NMR] spectroscopy, that these two diastereomeric sets of enantiomers exist in unequal amounts in "valnoctamide". Since isovaleramide possesses no optically active (chiral) stereocenters, it exists as a single, clearly definable molecular entity, with no

alternative enantiomeric or diastereoisomeric forms, and its experimentally determined pharmacological properties and profile are those of a single, pure molecular entity.

Doses of isovaleramide as relatively low as 30-
5 100 mg/kg IP in mice exhibit quantifiable CNS-depressant effects. Since the LD₅₀ value of isovaleramide is greater than 4000 mg/kg IP in mice, the therapeutic index of the compound appears to be on the order of at least 40. A comparable value for valnoctamide is on the order of
10 11.4. Roszkowski, A.P. and W.M. Govier, *Int. J. Neuropharmacol.* (1962) 1: 423-30. Valnoctamide is thus a barbiturate-derived higher homolog of isovaleramide which shares some of the properties of certain barbiturates, such as true hypnotic potential, which are
15 undesirable in this case.

In addition, certain α -branched isovaleramide derivatives, i.e., higher homologs, which are very closely structurally related to valnoctamide have been shown to exhibit a potential for producing hepatic
20 porphyria, a property also shared by certain barbiturates. See, for example, Schmid, R. and S. Schwartz, *Proc. Soc. Exptl. Biol. Med.* (1952) 81: 685-89; Case et al., *loc. cit.* (1953) 83: 566-68; Goldberg, A., *Biochem. J.* (1954) 57(1): ii; Goldberg et al., *Proc. Roy. Soc., Ser. B. (Biol. Sci.)* (1955) 143: 257-80; Talman et al., *J. Biol. Chem.* (1955) 212: 663-75; Talman et al., *Arch. Biochem. Biophys.* (1957) 66: 289-300; Goldberg, A. and C. Rimington, *DISEASES OF PORPHYRIN METABOLISM* (1962) Charles C. Thomas, pp. 175-99; Granick, S., *Ann. N. Y. Acad. Sci.* (1965) 123:1 88-97; Marks et al., *Biochem. Pharmacol.* (1965) 14(7): 1077-84; de Barreiro, O. C., *Biochem. Pharmacol.* (1965) 14: 1694-96; Hirsch et al., *loc. cit.* (1966) 15(7): 1006-08; Hirsch et al., *loc. cit.* (1967) 16(8): 1455-62; Schneck et al., *loc. cit.* (1968)
25 17(7): 1385-99; Schneck, D.W. and G.S. Marks, *loc. cit.* (1972) 21(18): 2509-518. In contrast, isovaleramide has no hepatic porphyria-inducing properties, presumably because of a lack of steric hindrance at the α position

to ready amide hydrolysis by liver enzymes. See Hirsch et al., *Biochem. Pharmacol.* (1966) 15(7): 1006-08; Hirsch et al., *loc. cit.* (1967) 16(8): 1455-62; Schneck et al., *loc. cit.* (1968) 17(7): 1385-99; Schneck, D.W. and G.S. Marks, *loc. cit.* (1972) 21(18): 2509-18.

Neodorm, a brominated higher homolog of isovaleramide (see Figure 1), also has been marketed as a sedative-hypnotic agent. See YEAR BOOK OF THE AMERICAN PHARMACEUTICAL ASSOCIATION, 1929 (1931) 18:154); *loc. cit.* 1931 AND 1932 (1934) 20,21: 154. Several reports erroneously identify Neodorm as α -ethyl isovaleramide; however, it is known that the α -brominated structure shown in Figure 1 in fact is the correct structure.

The activities of chemically defined agents intended to affect the central nervous system show an interesting lack of pattern. For example, derivatization (*N*-alkylation) of the nitrogen atom of the amide group has produced compounds such as *N,N*-diethylisovaleramide (A. Liebrecht, German Patent 129,967, issued 1902) which has been reported to have sedative activity and has been marketed as a sedative. Budavari et al. (Eds.), THE MERCK INDEX, 11th ed., page 824, no. 5122 (Merck & Co., 1989) (see "Valyl" in Figure 1). However, this compound is shown below in fact to exhibit CNS-stimulating, anxiogenic (that is, anxiety-inducing), and convulsant properties. Indeed, *N*-methylated amide derivatives can show either CNS-stimulating or -depressing properties, whereas *N*-ethyl and larger derivatives generally possess CNS-stimulating properties. Volwiler, E.H. and D.L. Tabern, *J. Am. Chem. Soc.* (1936) 58: 1352-54; Nelson et al., *J. Am. Pharm. Assoc., Sci. Ed.* (1941) 30: 180-82. This is analogous to the effect of addition or subtraction of methyl or methylene groups in other CNS agents such as catecholamine- and serotonin-like agents (F.W. Schueler (Ed.) MOLECULAR MODIFICATION IN DRUG DESIGN, ADVANCES IN CHEMISTRY SERIES No. 45 (1964) American Chemical Society, pp. 114-139), the barbiturates (Burger, A. (Ed.) MEDICINAL CHEMISTRY, Part II, 3rd ed.

(1970) Wiley-Interscience, pp. 1365-85), and other compound classes (Slater et al., *J. Pharmacol. Exptl. Therap.* (1954) 111:182-196), which can then exhibit either CNS-depressing or -stimulating properties.

5 Additional related compounds, valproic acid and valpromide, a higher homolog of isovaleramide (see Figure 1), have been used as antiepileptic (anticonvulsant) drugs. Gilman et al. (Eds.) GOODMAN AND GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 8th
10 ed. (1990) Pergamon Press, pp. 436-62. To the contrary, isovaleramide itself has no anticonvulsant properties.

Accordingly, previous studies demonstrate that there are no clearly discernible structure-function relationships which permit predictability of the effects
15 of experimental compounds upon the central nervous system. In particular, it is not possible to predict which alkylamide derivatives can provide therapeutically useful anxiolytic and sedative agents.

Summary of the Invention

20 The invention is directed to the use of isovaleramide as an active ingredient in pharmaceutical compositions which are mildly anxiolytic at low dosage levels and mildly sedative at somewhat higher dosage levels. Isovaleramide does not produce undesirable sedative
25 effects at the lower dosages, nor does it behave as a hypnotic, and it also is essentially nontoxic.

Surprisingly, a general pattern of activity among isovaleramide homologs has been discovered by the present inventors. For example, *n*-butyramide, a lower homolog of
30 isovaleramide, has no significant anxiolytic activity at doses in which isovaleramide induces anxiolysis, while valnoctamide, a higher homolog of isovaleramide, is about ten times more potent than isovaleramide as a sedative-hypnotic agent (see Example 2 below). Thus, lower
35 homologs of isovaleramide have a sedative activity that is too weak for clinical utility, whereas higher homologs

induce sedative-hypnotic effects which render the compounds unsuitable for over-the-counter use. In contrast, isovaleramide possesses an optimal balance of desirable pharmacological properties that render the compound particularly suitable for use as a mild psychotherapeutic agent for over-the-counter applications.

In accordance with one of its aspects, therefore, the present invention is directed to a use of an amount of isovaleramide in the preparation of an agent for use in a method to produce an anxiolytic effect in a subject in need of such treatment, wherein the amount of isovaleramide is effective to reduce anxiety without inducing excessive sedation. According to another aspect, the present invention is directed to a use of an amount of isovaleramide in the preparation of an agent for use in a method to facilitate sleep in a subject in need of such treatment. The invention also is directed to pharmaceutical compositions useful in the foregoing methods, especially oral compositions.

Brief Description of the Drawings

Figure 1 shows the chemical structures, generic and trade names, and uses of isovaleramide and structurally related known compounds.

Figure 2 shows the anxiolytic activity of isovaleramide and structurally related and control compounds derived from Vogel assay data from male rats and expressed as an anxiolytic index.

Figure 3 shows the general sedative effect of isovaleramide as opposed to valnoctamide, demonstrated by electronic measurements of drug-induced decreases in spontaneous locomotor activity in male rats.

Figure 4 shows the average relative percent increase in pentobarbital-induced sleep time for isovaleramide-treated mice over control mice.

Detailed Description of Preferred Embodiments

Pursuant to the present invention, isovaleramide is employed as a mild anxiolytic and, at higher doses, as a mild sedative agent. Thus, these two effects of isovaleramide can be separated and emphasized, according to the present invention, based on dosage level.

The ability of isovaleramide to exert these effects without undesirable side effects is a major feature of its utility. It therefore is important to distinguish between the various behavioral outcomes which can be experimentally determined as a result of administering agents which affect the central nervous system. Some of these behaviors and tests which assess them are as follows:

Anxiolytic Activity

An anxiolytic effect can be measured using either the exploratory behavior test or the Vogel Conflict Paradigm.

The exploratory behavior test, as described by Christmas, A.J. and D.R. Maxwell, *Neuropharmacology* (1970) 9: 17-29, and by Sepinwall, J. and L. Cook, *HANDBOOK OF PSYCHOPHARMACOLOGY* (1978) 13: 345-93, is conducted by measuring an increase or decrease of locomotor activity when the animal is placed in a novel or familiar environment. Anxiolytic agents increase exploratory behavior in a novel environment (because they reduce fear and anxiety), but they do not affect behavior in a familiar environment.

In one form of the test, wooden chambers (65 cm x 100 cm x 20 cm) with a wire-mesh floor and phototubes 3 cm from the floor are used. The phototubes are arranged in a grid and register the animal crossings (a measure of locomotor activity) through a break in the light path. The phototube output is fed to a computer (IBM) for data storage and analysis. Activity is quantified by counting the total number of crossings and the number of crossings per unit time.

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Sprague-Dawley male rats are injected intraperitoneally (IP) with either the test or control substance and are then placed into the activity chambers. They are observed for 1 hour. This will be a novel environment to them. Locomotor activity during the initial 5 minutes is increased, because of "disinhibition" by anxiolytic agents.

At the end of the first trial, the animals are removed and allowed to recover in the animal colony for 7 days, and are then placed in the same activity chambers for 1 hour on days 8 and 9. By this time, it is considered that the environment is familiar to the test animals. Injection of the test substance or control on day 10 and repetition of the experiment thus gives results in a familiar environment.

In the Vogel Conflict Paradigm, thirsty rats are given mild electric shocks through a metal water dispenser when they try to drink. The shock is unpleasant, but not severe enough to cause overt pain. When administered anxiolytic drugs, the rats lick the dispenser more often than controls (again, because of "disinhibition" due to reduction of anxiety). Assessment of anxiolytic effects is thus measured as an increased number of licks.

As described below, a modified form is employed of the standard Vogel assay described, for example, by Petersen, E.N. and J.B. Lassen, *Psychopharmacology* (1981) 75: 236-39. Animals (male Sprague-Dawley rats, 250-300 g) are deprived of water for 24 hours and then are placed into a clear Plexiglass box (45 cm x 25 cm x 20 cm) which rests on a grid of stainless steel bars. A circuit between the grid bars and the drinking spout is established with a silver electrode in a drinking bottle placed in the chamber. The animals are allowed to lick the water dispenser for 5 minutes without shock; those animals that fail to lick are excluded from further study.

The animals are returned to the home colony and are again deprived of water for 24 hours and replaced in the chamber and allowed to lick for 1 minute without shock. Then a 1 milliamp current (shock) is maintained for 4 min
5 at the drinking spout. Only the animals showing 50% suppression in licking behavior compared to the first session are included in further testing.

The test substances are injected intraperitoneally (IP) after this session, and 30 minutes later the animals
10 are again placed into the chamber. Again the animals are allowed to lick without shock for 1 minute and the shock is instituted for the remaining 4 minutes. The number of punished licks during this time is used to assess anxiolytic activity.

15 Both of the foregoing assays detect, demonstrate, and permit the quantification of anxiolytic activity. In the first assay, sedatives show no activity, whereas in the Vogel Conflict Test sedatives also give positive results.

Sedative Activity

20 Sedative effects can be measured by the prolongation of the time of barbiturate-induced sleep. In this assay, animals are injected IP with the test compound or control substance and 15 or 30 minutes later injected IP with
25 50 mg/kg pentobarbital sodium. The animals are then placed in a small Plexiglass chamber in a behavioral testing room and the latency to sleep and duration of sleep are recorded manually. The onset of sleep is defined as occurring when the animal loses the ability to
30 right itself when placed on its side (Irwin, S., *Psychopharmacologia* (1968) 13:222-257). The duration of sleep is defined as the interval occurring between loss and recovery of the righting reflex.

Hypnotic Activity

Hypnotic activity can be assessed by simply determining the doses of the test substances (administered by IP injection) sufficient to cause a loss of the righting reflex (see above) and an apparent loss of consciousness and responsiveness. This is considered to represent the "induction of sleep", and is clearly distinguishable from a sedative effect in which the experimental animals are merely slowed or rendered sluggish in their behaviors, but retain the righting reflex to some degree, as well as some measure of responsiveness.

Characteristics of Isovaleramide

The effect of isovaleramide in the assays for various CNS effects, combined with its lack of toxicity, make it an ideal mild anxiolytic and, at higher dosages, a mild sedative agent. For example, isovaleramide is useful in treating persons suffering from symptoms of stress and mild anxiety, including tension, restlessness, nervousness, inability to concentrate, irritability, over-aggressiveness and insomnia. Other conditions and therapeutic regimens which may benefit from the anxiolytic effects of isovaleramide include the treatment of the symptoms of smoking cessation, treatment of alcoholism and other substance abuse, premenstrual syndrome, menstrual discomfort, and hyperexcitability in children.

Figure 1 shows the structures of various known compounds which are structurally related to isovaleramide. Isovaleramide was initially prepared by the present inventors from extracts (i.e., ammoniated tinctures) of the underground parts of *Valeriana officinalis* L. (common name: valerian). Extracts of this plant and closely related species have been used historically as sedatives and antispasmodics. As indicated above, however, what was known heretofore about valerian extracts, and about the compounds shown in

Figure 1, would not have suggested that isovaleramide per se was an active agent or that the compound possessed properties recommending it for use in the preparation of a pharmaceutical formulation that produces an anxiolytic effect without inducing excessive sedation or, with a higher dosage of the active agent, that facilitates sleep in a subject needing such treatment.

For a formulation of the over-the-counter type, an oral route of administration is preferred and can be achieved via solid oral dosage forms such as enteric-coated tablets, caplets, gelcaps or capsules, or via liquid dosage forms such as syrups or elixirs. The indicated dosage of isovaleramide as an anxiolytic is on the order of 100-1000 mg per adult, preferably 100-500 mg per 60-70 kg adult. This amounts to about 1.5-20 mg/kg, preferably about 1.5-10 mg/kg. Unit solid oral dosage forms generally contain about 100-250 mg/tablet or capsule, which typically would be taken 1-2 at a time for a maximum of four times per day. Liquid formulations can also be employed with active ingredient compositions so as to provide 1-2 teaspoonfuls per dose. Furthermore, corresponding reduced dosage pediatric chewable and liquid oral dosage forms can also be developed. This compound can also be added to foods and beverages in the form of drops (with a dropper from a "concentrate" preparation) for oral administration. In addition, isovaleramide may also be formulated into chewing gum.

The isovaleramide active ingredient may also be administered by injection or other systemic routes, such as transdermal or transmucosal administration, for example, nasally, buccally, or rectally, via suppositories. However, oral administration is much more convenient and, hence, is preferred.

It is further understood that isovaleramide can be used in combination with other pharmaceutically active ingredients.

For use as a mild sedative to facilitate sleep, the dosage level is on the order of 500-1000 mg per typical

adult or about 10-20 mg/kg, generally taken 15-30 minutes before facilitation of sleep is desired. Oral dosages can be formulated in correspondingly higher concentrations or larger numbers of capsules or tablets can be taken. In this case, also, the isovaleramide may be simply added to foods or beverages consumed before retiring. Persons suffering from insomnia will benefit from these mild sedative effects.

In addition to the use of isovaleramide as a mild anxiolytic or a mild sedative for humans, isovaleramide may also be useful as a mild anxiolytic or mild sedative agent for domestic or domesticated animals in which excitation is undesirable, such as, but not limited to, cats, dogs, birds, horses, cattle, mink, poultry and fish. In such cases, the isovaleramide may be administered by injection or other systemic routes such as transdermal or transmucosal administration (for example, rectal administration via suppositories) or orally by addition to food or drink. The indicated dosage of isovaleramide per kilogram of body weight of such animals is about 0.15-20 mg/kg, preferably about 0.25-10 mg/kg, depending upon the species of animal and the route of administration. The indicated dosage of isovaleramide per kilogram body weight as a mild sedative for the animals is in the range of about 1.25-20 mg/kg, depending upon the species of animal and the route of administration.

The present invention thus contemplates a variety of pharmaceutical compositions containing isovaleramide, as active ingredient, that are suitable for oral, parenteral, transdermal, intranasal, buccal, or rectal administration. Although isovaleramide may be present as an incidental by-product in certain pharmaceutical formulations which are outside the scope of the present invention, the common feature of the claimed compositions is that isovaleramide is present in a standardized amount. That is, the claimed compositions contain a predetermined amount of isovaleramide to enable the

determination of the quantity of a particular composition required to achieve the isovaleramide dosage levels described herein.

5 The following examples are intended to illustrate, but not to limit, the present invention.

Example 1: Comparative In Vitro Cytotoxicity of Isovaleramide and Valepotriate Compounds

10 The valepotriate compounds valtrate, didrovaltrate and acevaltrate were compared with isovaleramide with respect to their effects on various human and murine tumor cell lines. The cells at log-phase growth were treated with various concentrations of test substances for 48 hr. Growth rates were then determined by directly counting cell numbers or by protein determination. EC₅₀
15 is the effective concentration of test substance that inhibited cell growth by 50%.

20 The results for isovaleramide as compared to the valepotriate compounds are shown in Table 1. As clearly shown, isovaleramide shows no cytotoxicity at concentration levels which are far beyond those at which the valepotriate compounds are highly toxic. Thus, the valepotriates are clearly demonstrated to be cytotoxic compounds (in the order valtrate > didrovaltrate > acevaltrate), whereas isovaleramide is non-cytotoxic.

Example 2: Comparative Effects of Isovaleramide on the CNS

Anxiolytic Activity

Isovaleramide and five other compounds were tested for anxiolytic activity in the Vogel Conflict Assay. Figure 2 shows the anxiolytic indexes of these test compounds, at various dose levels, which were derived from data obtained in the Vogel Conflict (anxiolytic) Assay using male rats. The "anxiolytic index" is defined as the ratio of the number of punished licks of test animals to that of control animals during a four-minute period. An anxiolytic index greater than one indicates anxiolytic ("calmative") activity. In practice, it is considered that significant "calmative" activity is observed when the anxiolytic index is greater than 1.5. An anxiolytic index less than 1.0 indicates anxiogenic (CNS-stimulant) activity. Anxiogenic compounds exhibit their effects on experimental animals as an increased state of vigilance, characterized by an increase in tension, fear, startle reflexes, etc.

Male Sprague-Dawley rats (250-300 g) were deprived of water for 24 hours before testing. The animals were allowed one five-minute period of drinking in the experimental chamber before receiving the test substances at the indicated doses. Thirty minutes later, the animals were returned to the chamber and allowed to drink for one minute without punishment. For the remaining four minutes, a shock was delivered to the drinking spout and the number of punished licks was recorded. Four or five animals were tested in each group.

Isovaleramide injected into rats IP at 500-1000 mg/kg showed an approximate three-fold increase in the number of punished licks over controls, demonstrating that it is clearly anxiolytic ("calmative") at these dose levels (Figure 2). ("VALIUM" (diazepam) injected in therapeutically effective doses elicits a ten-fold increase in the number of licks.) *n*-Butyramide, a lower homolog of isovaleramide (see Figure 1) and sucrose,

assayed as a control, showed no significant anxiolytic activity at comparable dose levels, whereas *n*-valeramide, a structural isomer of isovaleramide, and valnoctamide, a higher homolog of isovaleramide (both in Figure 1), did exhibit significant "calmative" activity at lower dose levels (250 and 50 mg/kg, respectively). These results emphasize the stronger hypnotic properties and tendencies of *n*-valeramide and valnoctamide, compared with isovaleramide. *N,N*-Diethylisovaleramide, the simple *N,N*-diethyl derivative of isovaleramide (Figure 1), although purported to have sedative properties, in fact exhibited CNS-stimulating (excitant) activity at 50 mg/kg (Figure 2) and was a potent convulsant in the rats at 250 mg/kg (data not shown in Figure 2). Thus, isovaleramide is the one compound from this group of test substances which has the best balance of pharmacologically desirable traits for a mild "calmative" (anxiolytic) agent.

Sedative Activity

Sedative activity elicited by a test substance can be observed directly in experimental animals by a decrease in spontaneous locomotor activity, for example, in open-field observation experiments. In such experiments, test animals are administered the test substance (usually by IP injection) and, after a suitable waiting period (usually 5-30 minutes), are placed into an open-field test chamber, which consists of a transparent plastic box with a clearly visible grid painted on the bottom (or floor). The numbers of excursions across the floor grids are then counted over a specified time period (usually 1-3 hours) and are subsequently compared with the numbers obtained from similarly treated saline controls. The number of grid crossings counted gives a direct measurement of the general activity level (measured as spontaneous locomotor activity) of each test animal in question. Sedative activity can be expressed as a percentage of the control level of spontaneous

locomotor activity as measured by grid crossings per unit time.

Direct observation experiments showed that naive observers could readily identify and distinguish isovaleramide-treated mice (at doses of 250 mg/kg IP) from saline-treated controls on the basis of general activity level alone, even without counting grid crossings. (Very astute observers also could distinguish test animals from controls at a dose level of 100 mg/kg IP.) At higher dose levels, for example, at 500 and 1000 mg/kg IP, the observed sedative effects were much more pronounced, with the experimental animals (male mice) clearly becoming progressively more heavily sedated as higher dosages were attained. At 1000 mg/kg IP, the isovaleramide-treated animals were clearly sedentary, with their coats very smooth and lustrous.

General sedative effects can also be measured electronically by recording drug-induced decreases in spontaneous locomotor activity in treated experimental animals, as shown in Figure 3. The data in Figure 3 clearly show that valnoctamide, the higher homolog of isovaleramide, is about ten times more potent than isovaleramide as a strong sedative-hypnotic agent. Valnoctamide was hypnotic to male rats at a dosage of 250 mg/kg IP, whereas at the same dose level isovaleramide decreased locomotor activity to a level comparable to that induced by valnoctamide at 25 mg/kg IP. Similarly, the decrease in locomotor activity induced by isovaleramide at 500 mg/kg IP is comparable to that induced by valnoctamide at 50 mg/kg IP. Thus, isovaleramide exhibits only about one-tenth of the potent (and, in this case, undesirable) sedative-hypnotic properties exhibited by valnoctamide at relatively low doses, and is therefore superior to it as a mild "calmative" agent.

The CNS-sedative activity of a drug substance can also be demonstrated by a prolongation of the sleep time of pentobarbital-induced sleep. In these assays,

experimental animals which are administered a hypnotic dose of a barbiturate (in this case, pentobarbital sodium, 50 mg/kg IP) will sleep for a longer period of time than controls (saline-treated animals) if a drug with sedative properties is co-administered. In our experiments, a sedative effect of isovaleramide could be detected and quantified at dose levels of 30-500 mg/kg IP administered to male Swiss-Webster mice (30-45 g) 30 minutes before receiving the pentobarbital at 50 mg/kg IP (see Figure 4). Thus, significant sedative effects on the CNS of the experimental animals, as shown by a significant prolongation of the pentobarbital-induced sleep time, could be demonstrated for isovaleramide-treated mice in the dose range of 100-500 mg/kg IP (see Figure 4).

Weak Hypnotic Activity and Potency

The hypnotic activity of isovaleramide was compared to that of valnoctamide, the aforementioned higher homolog of isovaleramide, by determining doses (administered to rodents by IP injection) sufficient to induce a loss of the righting reflex and an apparent loss of consciousness and responsiveness ("sleep induction"). Although valnoctamide was hypnotic (induced sleep) at doses of 250 mg/kg IP in male rats, isovaleramide was not hypnotic in male mice even at 1000 mg/kg IP. However, at the nearly toxic dose level of 2000 mg/kg IP in male mice, isovaleramide exhibited hypnotic activity. Thus, valnoctamide is a much more potent hypnotic than the weakly active isovaleramide, which produces hypnosis only in very large doses approaching toxic dose levels (i.e., greater than 1 g/kg).

Lack of CNS-Stimulating and Convulsant Activities and Other Acutely Toxic Effects

The potential for CNS-stimulating and convulsant properties was assessed for N,N-diethylisovaleramide (which is purported in the literature to be a sedative

agent) and for isovaleramide by administering appropriate doses of the test substances IP to rodents (male mice and rats). Although N,N-diethylisovaleramide is claimed to have sedative properties, it in fact showed potent CNS-
5 *stimulating* and *convulsant* properties at doses of 250 mg/kg IP in male mice and rats; at 500 mg/kg IP in male mice, N,N-diethylisovaleramide caused death following convulsive seizures. On the other hand, isovaleramide itself showed absolutely no CNS-stimulating
10 or convulsant properties at any dose tested in the range 10-4000 mg/kg IP in male mice. At the highest dose of isovaleramide tested (4000 mg/kg IP), some deaths resulted among the test animals (male mice) after sleeping for several days, apparently from respiratory
15 depression. This dose level was still below the LD₅₀ value of isovaleramide, however, since more than half of the experimental animals survived after sleeping for several days. Further testing of isovaleramide at still higher dose levels was precluded by its solubility limits
20 and the consequently overly large injection volumes required.

What Is Claimed Is:

1. The use of an amount of isovaleramide in the preparation of an agent for use in a method to produce an anxiolytic effect in a subject in need of such treatment, wherein said amount of isovaleramide is effective to reduce anxiety without inducing excessive sedation.

2. A use as claimed in claim 1, wherein said effective amount is in the range of 1.5-20 mg/kg body weight.

3. A use as claimed in claim 2, wherein said effective amount is in the range of 1.5-10 mg/kg body weight.

4. A use as claimed in claim 1, wherein the subject is human and the anxiolytic effect is useful for the treatment of mild anxiety, the symptoms of smoking cessation, alcoholism and other substance abuse, premenstrual syndrome, menstrual discomfort, and hyperexcitability in children.

5. A use as claimed in claim 1, wherein the subject is a domestic or domesticated animal and the anxiolytic effect is useful where excitation is undesirable.

6. The use of an amount of isovaleramide in the preparation of an agent for use in a method to facilitate sleep in a subject in need of such treatment, wherein said amount of isovaleramide is effective to facilitate sleep.

7. A use as claimed in claim 6, wherein said effective amount is in the range of 10-20 mg/kg body weight.

8. A use as claimed in claim 6, wherein the subject is human.

9. A use as claimed in claim 6, wherein the subject is a domestic or domesticated animal.

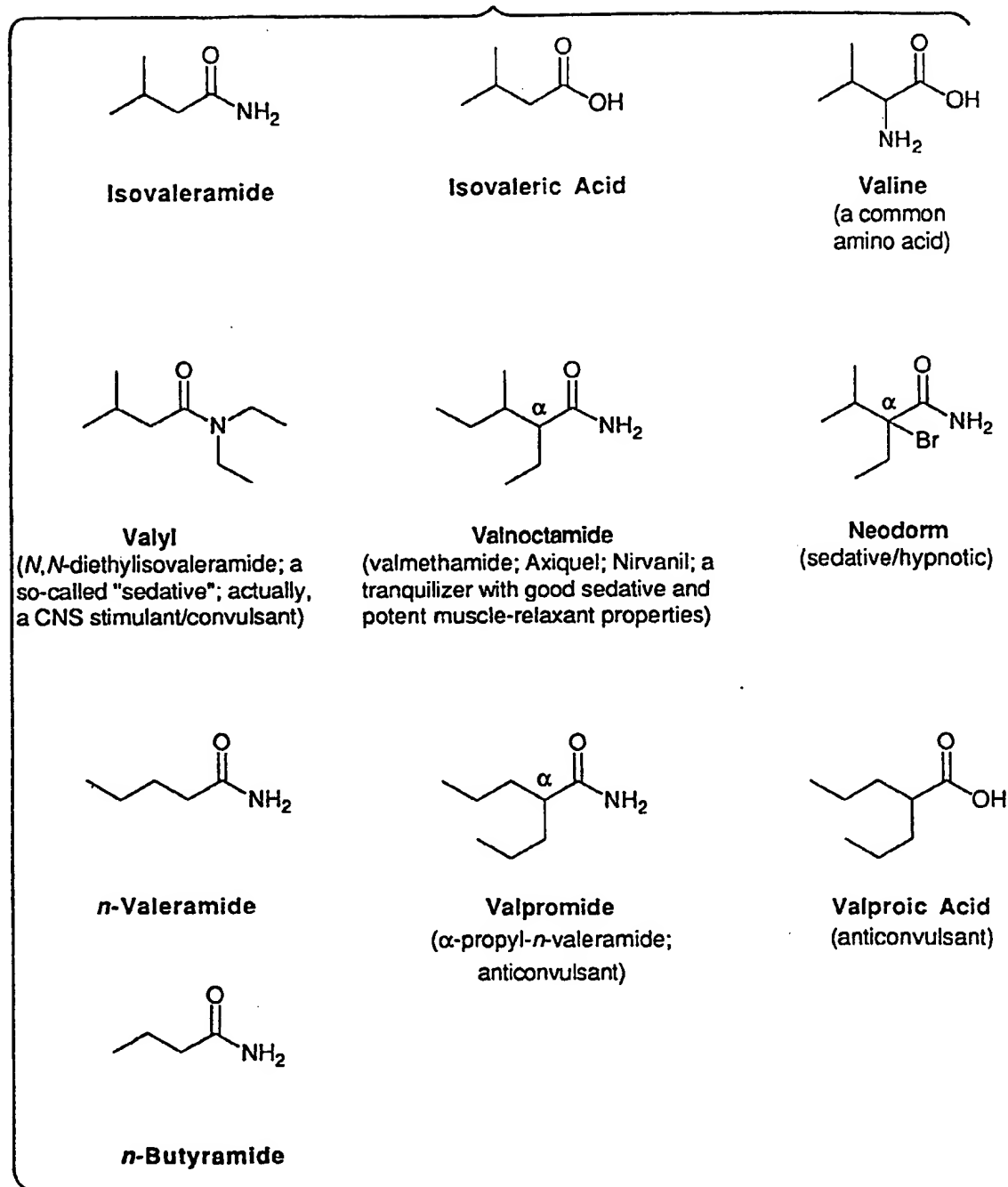
10. A pharmaceutical composition comprising a predetermined amount of isovaleramide in unit dosage form.

11. The pharmaceutical composition of claim 10, which is suitable for oral administration.

12. The pharmaceutical composition of claim 10, which is suitable for injection.

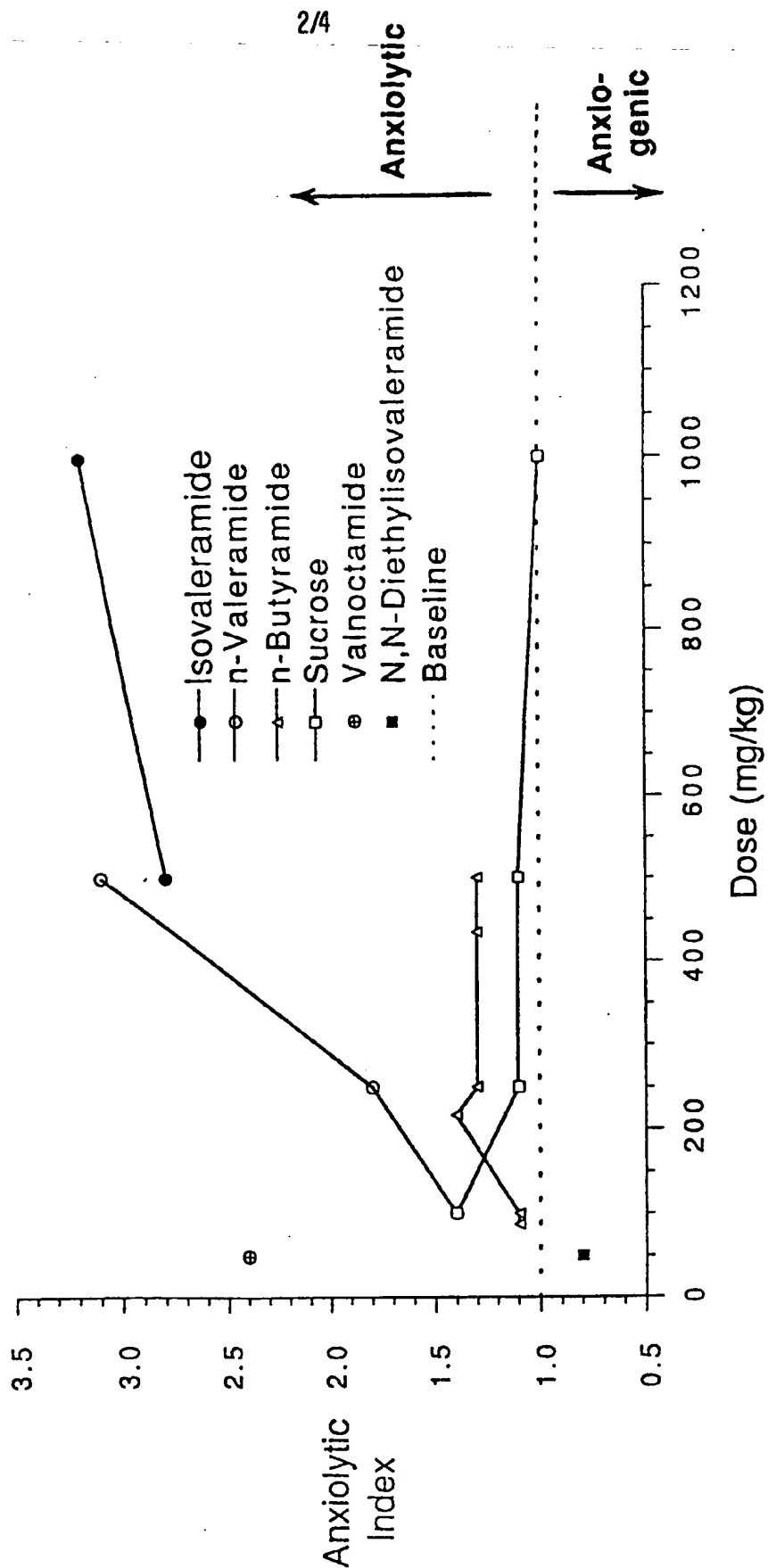
13. The pharmaceutical composition of claim 10, which is suitable for transdermal or transmucosal administration.

FIG. 1

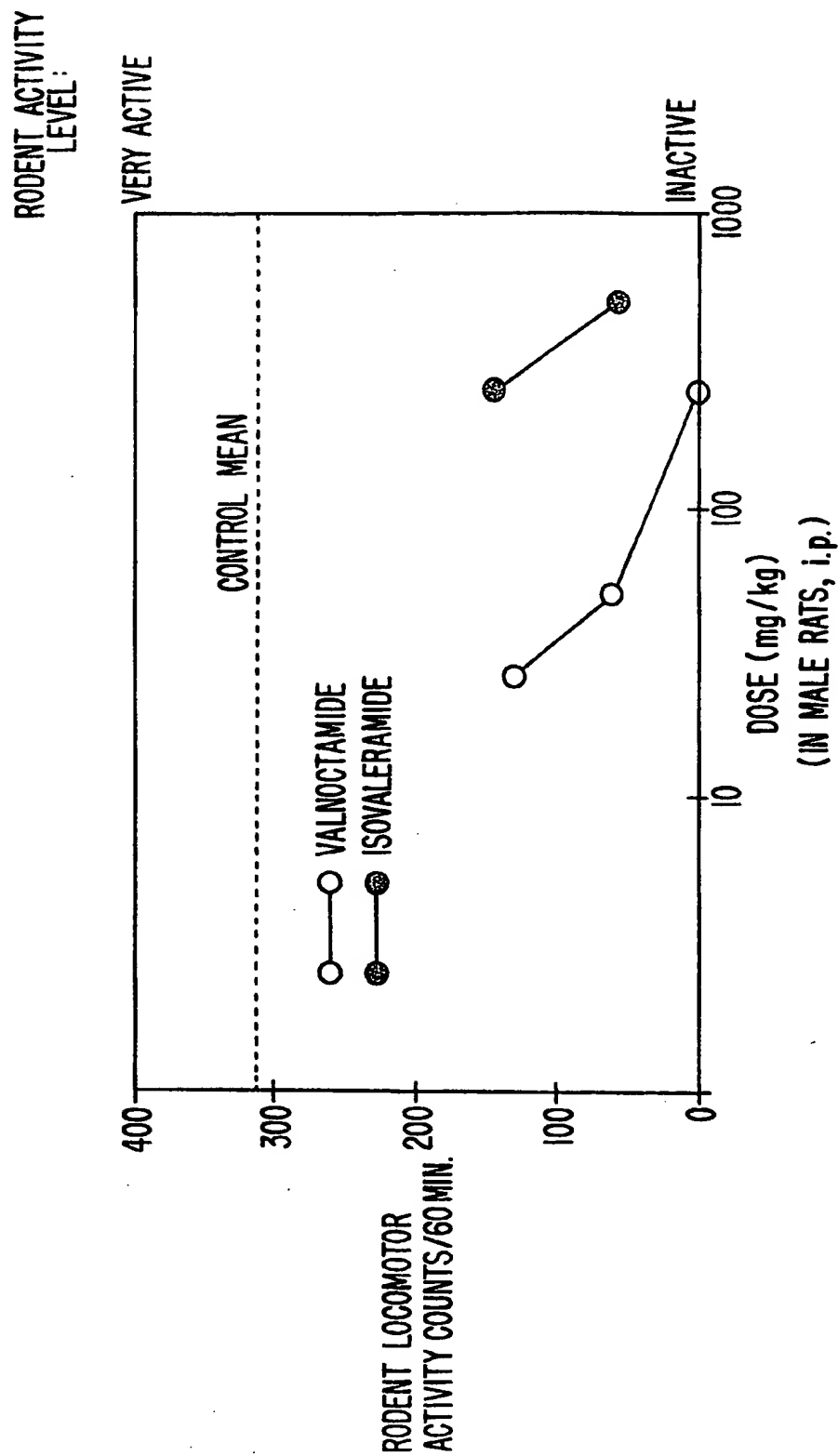


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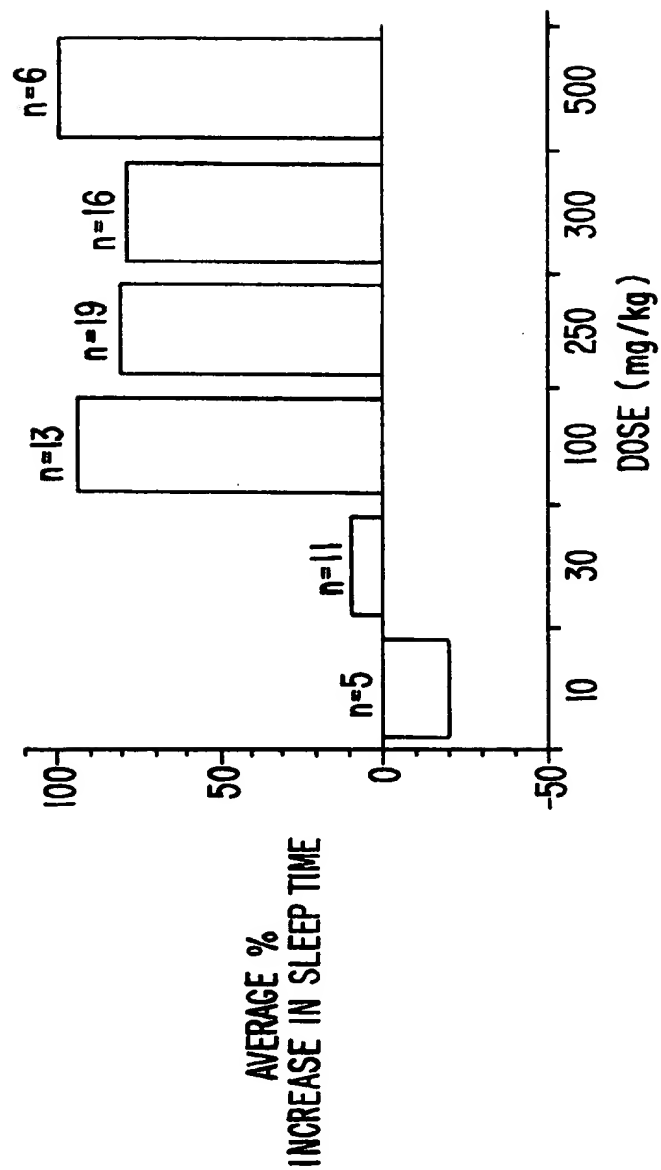
FIG. 2



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FIG. 3

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FIG. 4

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DEUT.MED.WOCHSCHR., vol.38, 1912 pages 945 - 947 'Pharmakologisches über Luminal oder Phenylethylbarbitursäure' cited in the application see page 945, left column, line 29 - line 45 ----	1-13
Y	NAUNYN SCHMIEDEBERG'S ARCH.EXP.PATHOL.PHARMAKOL., vol.186, no.37, 1937 pages 553 - 64 'Studien über die Wirkung substituierter Acetamide' cited in the application see page 553, line 15 - line 18 ----- -/-	1-13

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search

3 October 1994

Date of mailing of the international search report

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J.AM.CHEM.SOC., vol.58, 1936 pages 1352 - 54 'Some alkyl and aryl amides and ureides as hypnotics' cited in the application see page 1352, line 1 - line 14 ---	1-13
Y	CURR.THER.RES., vol.2, 1960 pages 144 - 47 'A clinical study on the use of valmethamide, an anxiety-reducing drug' cited in the application see page 147, line 7 - line 19 ---	1-13
Y	INT.J.NEUROPHARMACOL., vol.1, 1962 pages 423 - 30 'Behavioural and central muscle relaxant properties of 2-ethyl-3-methylvaleramide' cited in the application see page 428, line 18 - page 429, line 20 ---	1-13
T	'The Merck Manual, 15th ed.' , MERCK SHARP & DOME see page 2483 - page 2486 -----	1-13